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We have examined the effects of chemical stimuli on the putative olfactory organ of the squid ***Loligo opalescens***. Chemosensory capabilities were studied both at the behavioral level in living squid and at the individual receptor cell level using whole-cell voltage clamp. We found that low concentrations of certain test substances reproducibly elicited escape responses in living, restrained squid. Taking advantage of this link between chemoreception and motor pathways, we were able to map the region of highest chemosensitivity directly to the olfactory organ which is a small knob located in an ear-like flap lateral to each eye. 'Ablation' experiments, which were performed by treating the olfactory organ with a local anesthetic, further confirmed that the olfactory organ was the site of chemoreception. In examining isolated receptor cells, we found at least three morphologies, similar to those described by Emery in ultrastructural studies (1975). Voltage clamp experiments on the two most common cell types (pyriform and floriform) showed that they contain neuronal-like Na and K channels. We tested several chemicals including propyl-paraben, 4-amino-pyridine, methadone, and a snail hypobranchial gland extract, and found that every substance which elicited escape responses was also a potent blocker of K currents in the receptor cells. Since the different chemical block K channels via different mechanisms, we propose that for these substances, K channel block may be the relevant mode of signal transduction.

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EVIDENCE FOR CHEMORECEPTION

IN SQUID OLFACTORY ORGAN

William F. Gilly

Frank T. Horrigan

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Annual ONR Progress Report

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INTRODUCTION

Cephalopods are arguably the most intelligent and responsive of invertebrates. Squid, cuttlefish, and octopus are highly mobile predators which are largely visually oriented (Packard, 1972), but are also equipped with complex vestibular (Budelman, 1987), auditory (Hanlon & Budelman, 1987) and tactile (Wells, 1964; Wells & Young, 1975) capabilities. These modalities converge in the central nervous system to regulate specific motor outputs of great biological importance, e.g. escape jetting, chromatophore display, mating, homing, and long-distance migration (Messenger, 1983; Boyle, 1986).

Although an important body of work exists concerning the chemotactile sense in octopods (Graziadei, 1962; Wells, 1983; Wells, Freeman & Ashburner, 1965), virtually nothing is known about chemoreception of water-born molecules in these or other cephalopods (Boyle, 1986). Morphological studies have revealed putative chemosensory cells in several tissues, including an elaborate 'olfactory organ' (Graziadei, 1965; Woodhams & Messenger, 1974; Emery, 1975), but behavioral evidence for water-born chemoreception is limited to a single report on Octopus (Chase & Wells, 1984). In no case has a chemosensory function for the olfactory organ been demonstrated, nor have receptor cells been identified physiologically.

Squid have surprisingly received no behavioral or physiological attention in these areas, despite an existant anatomical literature on the olfactory organ (Emery, 1975) and on the central projections of axons from the putative receptor cells of this organ (Messenger, 1979). Afferent tracts lead to the motor centers which control swimming and escape jetting, and direct connections to the giant fiber pathway may occur. The anatomical basis thus exists for an important chemosensory element in the neural control of escape jetting in squid, a subject which itself has only recently come to be reinvestigated

(Otis & Gilly, 1990) after a hiatus of 30 years (Wilson, 1960).

Although the basis of water-born chemoreception in cephalopods and the behavioral outputs controlled by projections of the receptor cells into the central nervous system are important questions, an even more general set of questions concerns the primary mechanisms of chemosensory transduction at the level of the receptor cell membrane. Nothing is known about cephalopods in this regard, and very little is presently known even in those groups, such as insects (Kaissling, 1986), which have historically provided much of our understanding of the sensory physiology involved in olfaction. Much of the recent progress in this area has come from vertebrate chemoreceptor (taste and olfactory) cells dissociated from sensory epithelia of several species and studied with patch voltage clamp techniques. Two major themes on transduction mechanisms emerge from these studies.

First, vertebrate olfactory (Trotier, 1986; Maue & Dionne, 1987) and taste (Kinnamon & Roper, 1988) receptor cells are excitable and show a variety of conventional 'neuronal' ion channel types when studied in vitro with patch clamp techniques. The idea that activity of 'conventional' channel types can be directly modulated by odorant molecules stems from experiments with model membrane systems and channels reconstituted from olfactory epithelium which demonstrated apparently direct activation of K-selective (Vodyanoy & Murphy, 1983) and cation-selective channels (Labarca, Simon & Anholt, 1988) by nanomolar levels of odorant molecules. In the patch clamp studies, a decrease of voltage-dependent K conductance was found in taste cells in response to sour (citric acid) and bitter (quinine) stimuli (Kinnamon & Roper, 1988). In experiments on olfactory receptors, application of odorant stimuli resulted in ionic specific effects which could not be associated with any particular type of channel (Trotier, 1986).

Another class of transduction mechanisms is thought to involve

indirect activation of ion channels by intracellular cyclic nucleotides generated by odorant-dependent cyclases (Pace, Hanski, Salomon & Lancet, 1985; Sklar, Anholt & Snyder, 1986). Although the specific channels responsible have not yet been identified, micromolar levels of cAMP significantly decrease the apparent membrane resistance of excised patches from ciliary or somatic membrane of amphibian olfactory receptors (Nakamura & Gold, 1987). It has been suggested that cAMP activation of a relatively non-selective channel may be a basis of olfactory transduction, in analogy to the well established case for cGMP in visual receptor cells (Yau & Baylor, 1988). In principle other intracellular messengers could also act in this way.

What classes of odorants directly or indirectly activate normally silent channels in receptor cells, block normally open channels, or modulate voltage-dependent channels -- and where, and at what density, these channels are located on the receptor cells -- remain as major questions.

The first part of this paper addresses the chemosensory capabilities of squid. We provide behavioral evidence for detection of waterborn chemical stimuli by the olfactory organ. These experiments also demonstrate that the olfatory organ projections must converge on motor centers controlling escape jetting. The second part of this paper describes voltage clamp studies of chemosensory cells dissociated from the sensory epithelium of the olfactory organ. Like vertebrate chemoreceptors, those in squid are excitable and very 'neuronal-like' in their electrical properties. An important transduction mechanism may involve the blocking of voltage-controlled K channels by a variety of compounds, all of which act behaviorally to elicit escape responses when applied to the olfactory organ <u>in vivo</u>.

METHODS

Behavioral experiments. All experiments were carried out on living, but restrained, adult <u>Loligo opalescens</u> with techniques similar to

those described in Otis & Gilly (1990). Chemical stimuli were delivered by pressure ejection. 75-300 ms duration pulses delivered fluid at a rate of 1 ul/ms from a port 0.65 mm in diameter. Test substances were extruded only during pressure pulses; at other times the line was closed to prevent leakage out of or siphoning back into the supply line. The stimulating probe assembly also contained a pair of small Pt wires and an optic fiber to transmit electrical and visual stimuli. Pressure within the mantle cavity was monitored with a pressure transducer, and only responses which produced measurable pressure rises were scored as positive escape responses. Some animals were also videotaped in order to verify the spatial location of the stimulus plume relative to the olfactory organ in the mapping experiments.

Dissociation of receptor cells and voltage clamp experiments. The olfactory organ in Loligo, as in Lolliguncula (Emery, 1975), is a small knob located at the bottom of the cavity formed by an ear-like structure on the lateral aspect of the head (See Fig. 2 and Emery, 1975). The olfactory knob was excised and treated with non-specific protease (10 mg/ml Sigma Type XIV) in sterile-filtered sea water at 18-20^{o}C for 1 hr and then placed in a primary tissue culture medium as described by Brismar & Gilly (1987). Cells were obtained for study by simply dipping the piece of tissue into a drop of solution in the experimental chamber. Cells were utilized immediately after dispersal from olfactory knobs which had been cultured (15^{o}C) for no more than 48 hrs. After this time cells were still viable, but morphological characteristics (see below) became blurred.

Whole-cell voltage clamp experiments were carried out at 10^{o}C following standard procedures (Brismar & Gilly, 1987; Gilly & Brismar, 1989). The recording pipette was filled with an internal solution of 50 mM K glutamate, 50 mM KF, 400 mM tetramethylammonimum glutamate, 10

mM Na_2-EGTA and 10 mM Hepes (pH 7.4). The external artificial sea water (ASW) contained 450 mM NaCl, 10 mM KCl, 10 mM $CaCl_2$, 50 mM $MgCl_2$, and 10 mM Hepes (pH 7.6). The internal solution for studies on Na channels contained 220 mM NaCl,230 mM TEA, 10 mM Na_2- EGTA , and 10 mM HEPES. External Na channel solution contained 450 mM NaCl, 10 mM CsCl, 0 mM KCl, 10 mM HEPES, 10 mM $CaCl_2$ and 50 mM $MgCl_2$. Holding potential was -70 mV.

RESULTS

Identification of Chemoreception and Links to Motor Control.

Small volumes (< 100 ul) of sea water containing certain test substances at low concentration were routinely found to elicit escape responses when pressure ejected near the olfactory organ of a living squid. Figure 1A shows mantle pressure following delivery of a 300 ms pulse of blue food coloring (Schilling, McCormick, Inc., Baltimore, MD) at a 1:500 dilution. The pressure transients are similar to those following an electrical shock (Fig. 1B) or a strobe light flash (Fig. 1C) delivered to the same area between the olfactory organ and the eye. All 3 forms of stimulation thus lead to escape behavior involving high pressure jet propulsion. Delays for chemical and electrical stimuli are highly variable and always much longer than the delay for visual stimulation. These differences are in large part due to which motor system is first activated, the giant fiber pathway in the case of visual stimuli vs. a small fiber system in the other two cases (Otis & Gilly, 1990).

Blue food coloring was originally intended to serve as an inert tracking dye, and its efficacy in stimulating escape responses was surprising. This agent is a mixture of 2M propylene glycol, 20 mM Brilliant Blue FCF and 10mM propyl paraben (4-hydroxybenzoic acid propyl ester), an antifungal preservative. Each of these constituents was independently tested in behavioral experiments which showed unambiguously that propyl paraben was the only substance that could act

alone to stimulate escape responses.

In two different animals, a total of 15 trials with 1:500 blue food coloring (in sea water) produced 9 positive escape responses, whereas 11 trials with 50 uM Fast Green as a control yielded no responses. In a third animal 50 uM propyl paraben elicited escape jets in 10 of 17 trials, and 100 uM Brilliant Blue and 100 uM Fast Green were completely uneffective (4 trials each). Several experiments with propylene glycol were also negative.

Threshold for activity of blue food coloring was 1:1000 (1 of 7 trials), corresponding to a propyl paraben a concentration of 10 uM. Because of the method for scoring a positive reaction (high pressure escape response), this figure is an upper limit for detectability.

Localization of the Chemoreceptive Site to the Olfactory Organ.

Two approaches were taken to localize the area of chemoreception. The first was to spatially map sensitivity to a potent stimulus. With the stimulating probe positioned directly adjacent to the olfactory knob (a few mm away), a threshold pulse duration of 100 ms was observed for 100 uM propyl paraben to produce an escape response. The probe was then moved to a new site, and another estimate of threshold pulse duration was obtained. Figure 2 shows threshold duration at various positions; + indicates a response for the given duration, - indicates no response with the maximum duration used. Similar mapping experiments were carried out on 4 additional animals, and in every case, small displacements of the stimulus plume from the olfactory knob decrease the efficacy of a given stimulus, consistent with the proposition that the olfactory knob is a chemoreceptive organ which can influence the motor centers that control escape jetting.

b A second way of localizing chemoreception to the olfactory knob involved reversibly blocking function by treating this structure with a potent, short-acting local anesthetic to impair transduction in the

receptor cells and/or afferent transmission in sensory axons of the olfactory nerve. Anesthetic treatment was performed on one side of the head; the contralateral untreated organ served as a control.

Figure 3 shows results obtained in such an experiment. High-pressure escape jets are produced by every stimulus (150 ms pulse of 100 uM propyl paraben) on both the control and test organs prior to the application of 10 ul of 0.5 mM dibucaine (in natural sea water) to the right olfactory knob. Function on this side was abolished for approximately 30 min, after which recovery occurred. The control side remained responsive throughout the period of dibucaine block. These 'ablation' experiments thus lend additional support to the idea that the olfactory organ mediates detection of propyl-paraben in the ambient sea water. Presumably this is a noxius stimulus, as evidenced by the strong escape response.

Detection of Other Substances by the Olfactory Organ

Experiments similar to those described above in conjunction with testing the efficacy of blue food coloring and propyl paraben as activators of escape jetting were carried out with a variety of other substances. Table I summarized results with several agents that consistently produced positive responses. These particular substances were focused on because of their ability to block voltage dependent K channels, a property which they share with propyl paraben (See also below). Spatial mapping of sensitivity to methadone also yielded results in agreement with those described above for propyl paraben, and presumably the olfactory organ mediates detection of all of these substances.

Positive results were also obtained with a 1:10 dilution of a crude extract of the hypobranchial gland from Calliostoma canilicula-tum, a subtidal snail which produces a noxius defensive secretion in response to stimulation by contact with predatory starfish (Smaby, 1988). In a single animal 11 out of 18 trials with this extract

elicited escape jets (0 out of 12 for sea water controls). Mapping experiments were not carried out in this case, however.

Other substances tested did not produce escape jets, and these results are summarized in Table II. Negative results reported here cannot be taken to imply that the squid cannot detect any of these substances, however, because our assay for a positive response probably rules out all but the most noxious stimuli. Upon exposure to some of the substances in Table II animals would often appear to display a change in respiratory rhythm, but quantitation of such responses was not attempted.

Morphological Identification of Receptor Cell Types.

Figures 4A-F show examples of freshly dissociated olfactory knob cells. At least three kinds of putative chemoreceptor cells are recognizable. The most common type (Fig. 4A,B) is pyriform with a long axonal process (**ax**) arising from the pole containing the nucleus (**n**) and a spike-like cilium which projects from the opposite pole (arrow). Most of the cell body appears to be filled with granular material or cristae, and the bulk of the cell's 'interior' is thought to be an invaginating extracellular cavity filled with cilia (Emery, 1975). These cells are undoubtedly equivalent to the 'Type 4' chemoreceptor cells described on ultrastructural grounds by Emery (1975). The inset to Fig. 4B is taken from his paper and illustrates this receptor type. A low-power scanning electron micrograph of an aldehyde-fixed pyriform receptor is shown in Fig. 6G; the spike-like cilium is evident.

Another receptor cell type is floriform (Figs. 4C,D). This type also displays a prominent axonal process, but in this case a long, thin neck extends from the cell body and is crowned by a swelling covered with non-motile cilia arranged in petaloid fashion (arrow). A close correspondence to Emery's Type 2 (Fig. 4C) or Type 5 (Fig. 4D)

receptor is apparent. Figures 4H,I are scanning electron micrographs of a fixed floriform receptor.

A third, less common receptor may be a modified pyriform type based on the presence of a 'spike'-cilium on the apical pole (Figs. 4E,F). Unlike the pyriform receptors described above, however, this type is elongate with a constriction of varying severity between the cell body proper, which contains the nucleus, and the apical portion which is filled with the granular material. This type of receptor appears equivalent to Emery's Type 3 (Fig. 4G).

Neuronal-Like Sodium and Potassium Currents in Receptor Cells

Voltage clamp experiments using the `whole-cell' recording mode of patch voltage clamp were carried out on both pyriform and floriform receptor cells dissociated from the olfactory knob. Figure 5A shows Na currents (obtained by TTX subtraction) recorded for voltage steps ranging from -40 to + 70 mV in a pyriform receptor bathed in normal artificial sea water and internally dialyzed with a high-sodium solution. The rapidly activating, transient currents show a definite reversal potential of ~+45 mV (Fig. 5B). In other experiments (not illustrated) only inward currents flow if Na ions are omitted from the internal solution. Inactivaton is steeply voltage-dependent between -50 and 0 mV and is half-maximal at ~ -25 mV.

All of the above properties are those expected for`conventional' neuronal-like Na channels, and additional analyses (not illustrated) indicate that the TTX-sensitive Na current in pyriform receptors is very similar to that found in squid giant axon and giant fiber lobe (GFL) neurons (Gilly & Brismar, 1989). Figures 5C,D demonstrate that similar Na currents also exist in floriform receptor cells, but these are much smaller than the corresponding currents recorded in pyriform cells.

Other experiments were designed to study voltage-dependent K currents (i.e., Na-free solutions with TTX present), and a series of

currents recorded between -20 and +70 mV from another pyriform receptor cell is illustrated in Fig. 6A. Outward currents activate with a marked, voltage-dependent delay, and inward tail currents flow upon termination of the pulses. Analysis of reversal potential indicates that these currents are carried by K-selective channels (not illustrated), and the kinetic properties (Fig. 6A) and voltage-dependence of the peak K conductance (Fig. 6C) are very similar to analogous data obtained in squid giant axon or GFL neurons (Llano & Bookman, 1986). As in squid GFL cells, the K currents in pyriform receptors inactivate to a large degree during a long depolarization (Fig. 6B).

Floriform receptor cells also have voltage-dependent K currents that are qualitatively similar to (but consistently smaller than) those in pyriform cells (Figs. 6D-F). In this case, however, the kinetics of inactivation are slightly more rapid than than those in pyriform cells.

Pharmacological Block of Potassium Currents in Receptor Cells

4-aminopyridine (4AP) is both a classical blocker of (closed) K channels (Yeh, Oxford & Narahashi, 1976) and a potent activator of escape jetting in living squid. The effects of this substance were therefore studied on K currents in dissociated receptor cells. Figure 7A shows Na and K currents at +30 mV in a pyriform receptor before and after (**+4AP**) bath application of 5 mM 4AP. Outward K current is reduced by roughly 50% (with no obvious change in time course), but there is no detectable block of inward Na current. Figure 7C illustrates analagous results from a floriform receptor cell. 4AP thus selectively blocks K currents in both types of receptor cells.

Methadone is a substance which is known to block open K channels (Horrigan, 1990) and which also elicits strong escape jets in behavioral experiments when it is applied to the olfactory organ. Figures 7B,D show the effect of 500 uM methadone on Na and K currents in a

pyriform and floriform cell, respectively. This drug, unlike 4AP, is a potent blocker of both Na and K currents. Non-selective block of both currents was also found in both types of receptor cells for propyl paraben (not illustrated), a substance which also reliably evokes escape responses in living squid.

DISCUSSION

The squid's 'olfactory organ' was not named for its chemosensory capabilities (which were at that time untested) but for its location and morphology (Emery, 1975). It is ideally located to detect water-born chemicals during the inspiratory phase of respiration and is coated with mucus and cilliated epithelial cells which may help to capture and concentrate chemical stimuli.

The putative olfactory receptor cells themselves are reminescent of olfactory cells in both vertebrates (Troiter, 1986) and invertebrates (Kaissling, 1986) with unipolar projections to the central nervous system. Their morphology shows the classic pear-shaped cell body with a narrowed stalk and a ciliated knob facing out into the external mileau. Like other chemosensory cells, squid olfactory cells contain voltage gated Na and K channels. Recent whole cell current clamp experiments show that squid olfactory cells are electrically excitable and capable of firing repetitive action potentials. (Data not shown).

Our work represents the first behavioral and electropyhsiological studies performed on squid olfactory organ. We find that the 'olfactory organ' of squid is indeed a chemosensory structure, at least capable of sensing noxious water-born chemicals and translating chemosensory information via higher pathways into an appropriate escape response. Olfactory input to the squid's major defense system (escape jetting) could increase survivability in nocturnal or low visibility situations as well as toxic or heavily polluted waters.

Propyl-paraben, a component in blue food coloring, elicits strong

escape responses in living squid, and blocks K currents in isolated voltage clamped olfactory cells. Assuming that blocking K channels would depolarize the receptor cell causing it to become hyperexcitable and/or increase its spontaneous firing rate, we tested the potassium channel blockers 4AP and TEA, and found that they also elicit escape responses. Voltage clamp experiments show that 4AP blocks only K channels. Classically, TEA blocks only K channels but voltage clamp experiments using TEA are not yet completed. It is likely that TEA will be similar to 4AP based on each chemical's ability to block K channels in other systems (Yeh, Oxford & Narahashi, 1976; Hille, 1984).

Methadone and propyl-paraben elicit escape responses and block both Na and K currents in isolated olfactory receptor cells. Although untested, it is possible that an escape response could be elicited by a change in spontaneous activity- whether it be an increase or a decrease in activity.

Interestingly, 4AP, propyl paraben, TEA, and methadone, have very different chemical structures and mechanisms for K channel block, but all of these substances elicit escape responses in squid. We propose that that K channel block itself may be important for signal transduction.

Without disregarding the possibility that block of Na channels can also lead to signal transduction, one can also imagine that Na channels in squid olfactory receptor cells may be spatially arranged in a fashion similar to taste receptor (Kinnamon & Roper, 1988) where in vivo tight junctions prevent exposure of specific channel populations to the external media. It will be interesting to conduct mapping experiments to test if ion channels are localized to specific regions in squid olfactory receptor cells.

There are many activities besides escape and avoidance where

chemoreception may be important to squid (ie. mating, migration, and feeding). Future experiments could make use of other behavioral assays such as respiratory rate or mating behavior for identifying physiologically important substances. These substances in turn would be used to investigate other mechanisms of chemosensory transduction in squid olfactory cells.

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TABLE 1

Chemicals which elicit positive escape responses in squid.

% Positive Responses and Number of Trials ()

Squid	Control	TEA 20mM	4AP 20mM	Methadone 1mM	Methadone 500uM
15Jan1	0 (9)	71 (7)	100 (1)	---	---
15Jan2	4 (26)	92 (12)	100 (4)	---	---
19Jan1	0 (8)	50 (6)	---	---	---
19Jan2	0 (20)	88 (8)	---	---	---
22Jan1	0 (16)	20 (10)	---	83 (6)	---
16May1	10 (21)	---	---	---	80 (10)
27May1	0 (12)	---	---	---	64 (14)

TABLE 2

Chemicals which do not elicit escape responses in squid.

% Positive Responses and Number of Trials

Chemical	%Positive	#of Trials	#of Animals
5mM Isethionate	0	(3)	1
5mM Betaine	0	(13)	1
5mM Menthol	0	(2)	1
Egg Jelly Extract	0	(6)	1
ASW pH 5	0	(9)	1
20mM Ba^{+2} ASW	11	(9)	1
20mM TMA	13	(18)	3

FIGURES

Figure 1. Chemical, electrical, and visual stimuli elicit escape responses in living restrained squid. Changes in squid mantle pressure reflect escape jetting of the squid in response to **A.** blue food coloring, **B.** electrical shock, and **C.** strobe light flash.

Figure 2. Mapping experiments on the squid's head using either propyl paraben or methadone show that the olfactory organ is the site of high chemical sensitivity.

Figure 3. Application of the local anesthetic betaine to the olfactory knob reversibly abolishes chemosensitivity to propyl paraben.

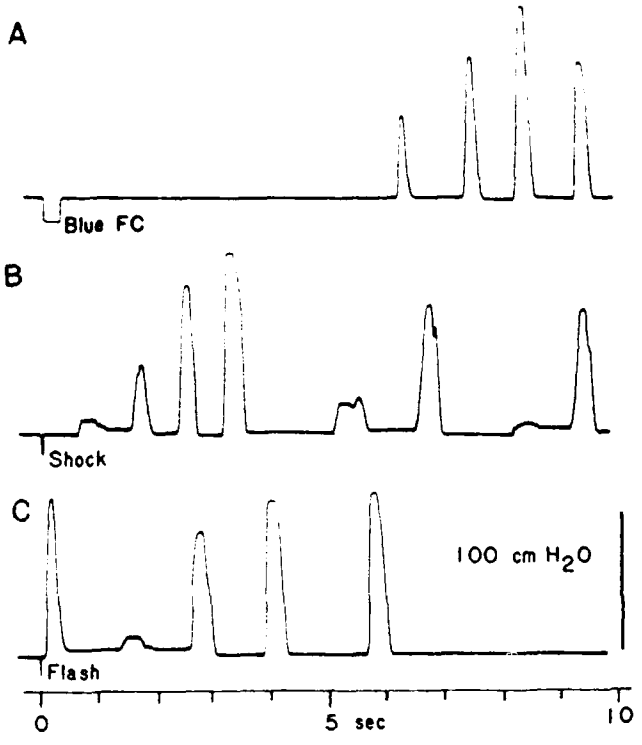
Figure 4. Olfactory receptor cells display at least 3 distinct morphologies. **A & B** are light microscopy of acutely isolated pyriform cells, **C & D** are floriform cells, and **E & F** are a modified pyriform cell. Drawings in inserts are from ultrastructural studies by Emery (1975). **G-I** are SEM micrographs of a pyriform cell, a floriform cell, and an enlarged floriform knob respectively.

Figure 5. Neuronal-like Na channels are found in both pyriform and floriform olfactory receptor cells. **A & C.** Whole cell TTX subtracted Na currents are from a pyriform and floriform cell respectively. Note the different scale bars for each. **B & D.** Current-voltage relationships were obtained by plotting the peak currents in A and C. The +45 mV reversal potential is close to the Nernst potential for these solutions.

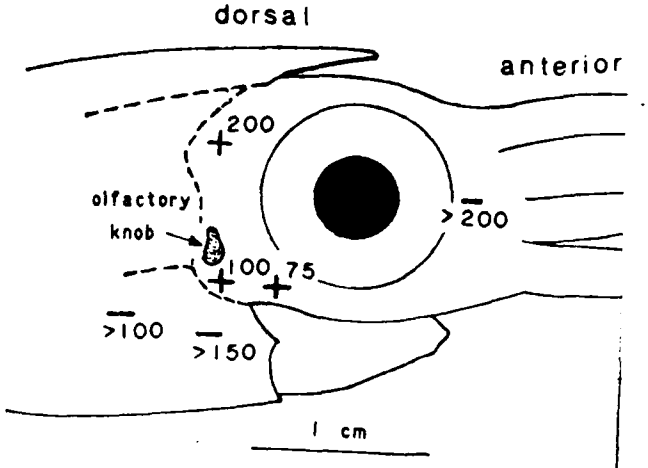
Figure 6. Delayed rectifier K currents are similar in pyriform and floriform receptor cells. **A & D.** Outward K currents in response to 30 ms pulses from -20 to +70 mV are shown for both a pyriform (A) and floriform (D) receptor. **B & E.** K current inactivates during the 300 ms pulse to +70 mV in the same cells as A & D. K channels in both cell types activate with similar kinetics as shown by the plot of relative conductance versus voltage in **C** and **F**.

Figure 7. Effects of channel blockers on pyriform and floriform receptor cells. Inward Na current is unaffected by 20 mM 4AP but outward K currents in **A.** pyriform and **C.** floriform cells are blocked by about 50%. **B & D.** Methadone blocks both channel types in pyriform and floriform cells respectively.

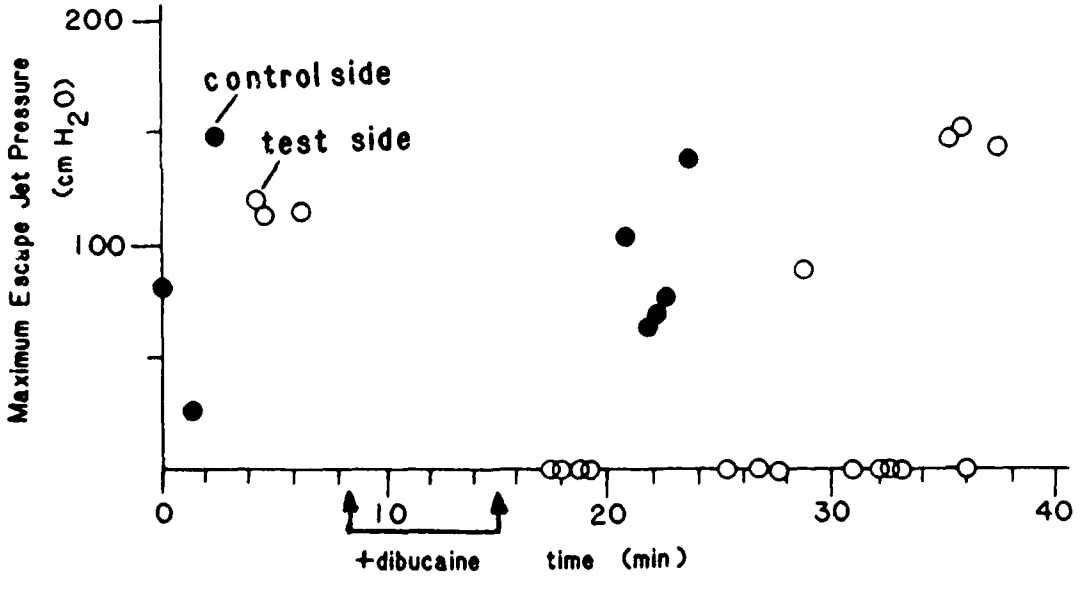
1 Chemostimulation of escape response

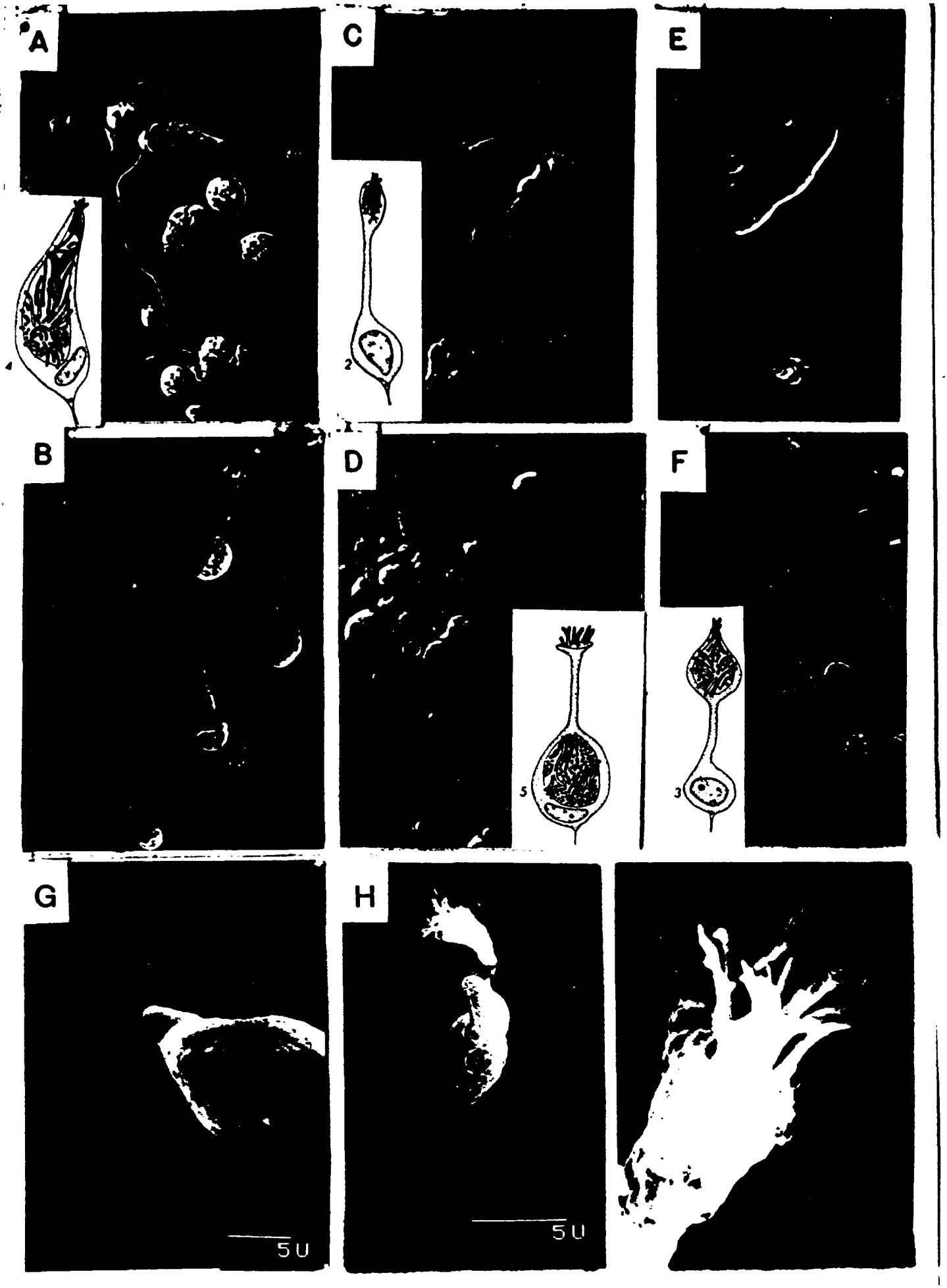


2 Olfactory organ is site of high chemosensitivity



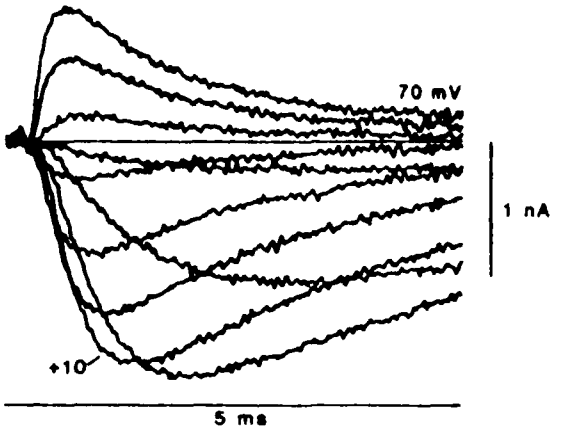
3 Application of local anesthetic to olfactory knob reversibly abolishes chemosensitivity



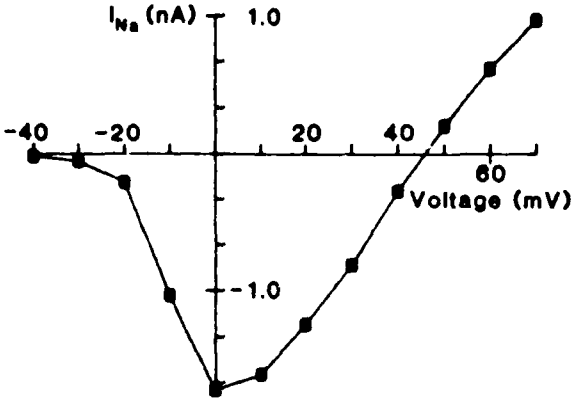

A
C
E
B
D
F
G
H
2
5
3
5U
5U

5

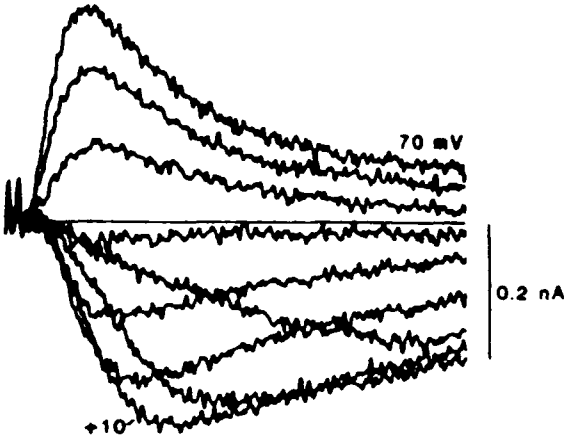
A


70 mV
1 nA
+10
5 ms

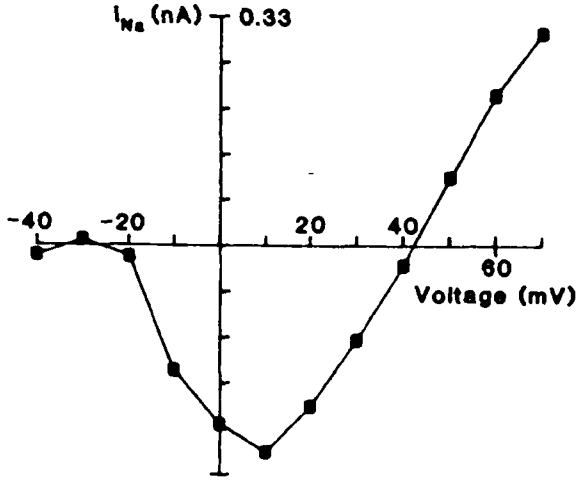
B


I_{Na} (nA)
1.0
-40
-20
20
40
60
Voltage (mV)
-1.0

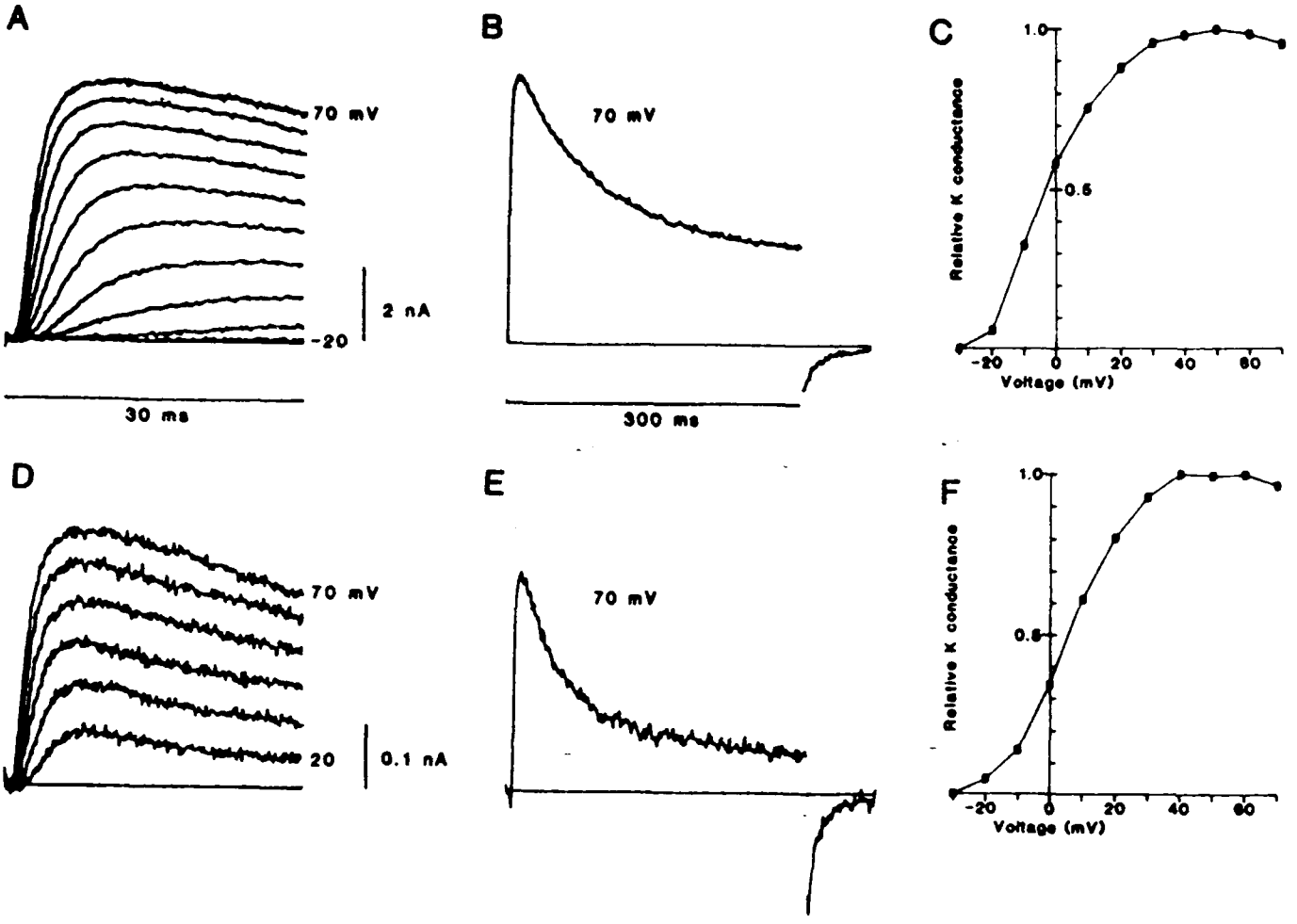
C


70 mV
0.2 nA
+10

D


I_{Na} (nA)
0.33
-40
-20
20
40
60
Voltage (mV)

6


A
70 mV
-20
2 nA
30 ms
B
70 mV
300 ms
C
1.0
0.5
Relative K conductance
-20
0
20
40
60
Voltage (mV)
D
70 mV
20
0.1 nA
E
70 mV
F
1.0
0.5
Relative K conductance
-20
0
20
40
60
Voltage (mV)

7